European Journal of Pharmaceutical Sciences 51 (2014) 157–164

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

In vivo pharmacokinetics, biodistribution and antitumor effect of amphiphilic poly(L-amino acids) micelles loaded with a novel all-trans retinoic acid derivative

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ARTICLE INFO

Article history:
Received 3 February 2013
Received in revised form 16 September 2013
Accepted 18 September 2013
Available online 26 September 2013

Keywords:
Poly(L-amino acids)
Micelles
Pharmacokinetics
Biodistribution
Antitumor effect
4-Amino-2-trifluoromethyl-phenyl retinate (ATPR)

ABSTRACT

Poly(amino acid)s are well-known as biodegradable and environmentally acceptable materials. In this study, a series of poly(L-aspartic acid)–b-poly(L-phenylalanine) (PAA–PPA) compounds with different degrees of polymerization were used to prepare copolymer micelles for a poorly water-soluble drug 4-amino-2-trifluoromethyl-phenyl retinate (ATPR, a novel all-trans retinoic acid derivative) and *in vivo* pharmacokinetics, biodistribution and antitumor efficacy of ATPR delivered by PAA–PPA micelles were evaluated. The area under the plasma concentration time curve $AUC_{0\to\infty}$ of ATPR-loaded PAA20PPA20 micelles was 2.23 and 1.97 times higher than that of ATPR solution and ATPR CrmEL solution, respectively; In addition, the mean residence time (MRT) was increased 1.67 and 1.97-fold, respectively and the total body clearance (CL) was reduced 2.25 and 1.98-fold, respectively. The biodistribution study indicated that most of the ATPR in the ATPR-M group was distributed in the liver and there was delayed liver aggregation compared with the ATPR solution and ATPR CrmEL solution groups. Furthermore, the antitumor efficacy of ATPR-loaded PAA20PPA20 micelles was demonstrated in *in vivo* antitumor models involving mice inoculated with the human gastric cancer cell line SGC-7901. At the same dose of 7 mg/kg, the ATPR-loaded micelles group demonstrated a better tumor growth inhibition and induced differentiation than the groups given ATPR solution and ATPR CrmEL solution. Therefore, the ATPR-loaded PAA–PPA micelles appear to be a potentially useful drug delivery system for ATPR and suitable for the chemotherapy of gastric cancer.

1. Introduction

The era of cancer chemotherapy began in the 1940s with the discovery of nitrogen mustard, a chemical warfare agent, as an effective treatment for cancer. Over the years, many antitumor drugs have been developed (Shewach and Kuchta, 2009). However, the unspecific drug disposition within the body has resulted in a limited antitumor efficacy and the production of severe side effects in sensitive healthy tissues. In addition, the short half-life and poor water-solubility of these newer agents is the main obstacle to their clinical application. Consequently, in recent decades, considerable attention has been focused on the development of novel drug delivery system for antitumor drugs. Polymeric micelles (PM) are widely used in novel drug delivery systems and PM are formed through the self-assembly of amphiphilic polymers in aqueous media. A hydrophilic block, as the outer shell, is responsible for a reduced uptake by the reticulo-endothelial system which results in the prolonged circulation of the micelles *in vivo* (Gref et al., 1994), and the hydrophobic block as the inner core, is responsible for the high loading capacity, stability and drug sustained-release of the drugs used. Due to their particle size, PM in solid tumors exhibit an enhanced permeability and retention (EPR) effect (Maeda et al., 2000; Torchilin, 2011). In addition, the PM system can reduce the P-glycoprotein (P-gp) efflux effect due to the alterative drug internalization route and subcellular localization (Mikhail and Allen, 2009; Kedar et al., 2010). Because of their small particle size, targeting ability, stability, long circulation and ease of production, PM have received in recent years growing scientific attention as an efficient drug carrier in recent years.

Poly(amino acid)s are well-known as biodegradable and environmentally acceptable materials. Earlier studies focussed on the poly(amino acid)-drug conjugate, poly(amino acid)-other carrier materials conjugate or poly(amino acid)/other carrier materials complex and the amino acid copolymer (Lalatsa et al., 2012; Tang et al., 2012). Amino acid copolymers, especially amphiphilic copolymers (with hydrophilic amino acids as the hydrophilic block, and hydrophobic amino acids as the hydrophobic block), can form micelles (Kidchob et al., 1998; Kim et al., 2009), fibers or vesicles (Holowka et al., 2007; Sun et al., 2007) through self-assembly.

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http://dx.doi.org/10.1016/j.ejps.2013.09.016

ATPR (4-amino-2-trifluoromethyl-phenyl retinate), a novel ATRA (all-trans retinoic acid) derivative, was prepared using ATRA and trifluoromethoxyphenyl agents as the starting materials by condensation with DCC and DMAP. ATPR exhibited superior differentiation-inducing activity on SGC-7901, BEL-7402, HT-29, and MDA-MB-231 cell lines, etc. (Hong et al., 2011; Shen et al., 2009; Wang et al., 2013a,b). However, the instability, poor water-solubility and short half-life *in vivo* of ATPR made the preparation of a commercial formulation difficult.

Poly(L-aspartic acid)–b-poly(L-phenylalanine) (PAA–PPA) was synthesized and its potential for the preparation of copolymer micelles with a poorly water-soluble drug (ATPR) was investigated by us in an earlier study (Tang et al., 2012). The ATPR-loaded PAA–PPA micelles (ATPR-M) had a narrow size distribution, low zeta potential, high drug-loading capacity and good stability and PAA–PPA was safer than Tween-80 and Cremophor EL (CrmEL) as an injectable pharmaceutical adjuvant for ATPR. The novel amphiphilic amino acid copolymer can be considered as a potential injectable delivery system for ATPR. Thus, ATPR-M was selected for further investigation and the *in vivo* pharmacokinetics, biodistribution and antitumor activity of ATPR-M were assessed and compared with ATPR solution and ATPR CrmEL solution as references.

2. Materials and methods

2.1. Materials

A series of PAA–PPA: PAA20PPA5, PAA20PPA10, PAA20PPA20, PAA20PPA30, PAA40PPA5, PAA40PPA10, PAA80PPA5, PAA80PPA10 (the digits represent the degree of polymerization of PAA or PPA) were synthesized by our lab group as described in a previous study (Tang et al., 2012) and their chemical constitution was shown in Fig. 1. ATPR was synthesized as described by previous study and its chemical structure is shown in Fig. 2 (Shen et al., 2009). Its purity was 99.73% as confirmed by HPLC. Acetonitrile and methanol used as the mobile phase in high-performance liquid chromatography (HPLC) were purchased from Tedia Company Inc (HPLC grade, Tedia Company Inc, USA). CrmEL was purchased from Sigma–Aldrich (Shanghai, China) while the Alkaline phosphatase (ALP) assay kit was obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other reagents were of analytical grade and used without further purification.

2.2. Cell line

The human gastric cancer cell line SGC-7901 was provided by the Cell Bank of Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China).

2.3. Animals

Sprague–Dawley (SD) rats and specific pathogen-free (SPF) male BALB/cA nude mice (5–6 weeks old) were obtained from Shanghai Slac Laboratory Animal Co. Ltd. (China).

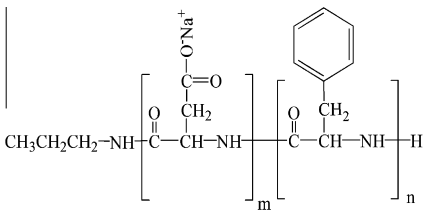

Fig. 1. Structures of PAA–PPA.

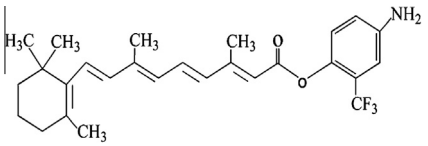

Fig. 2. Chemical structure of ATPR.

All the animals were allowed free access to food and water. All the animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health.

2.4. Synthesis of PAA–PPA

PAA–PPA was synthesized by ring-opening polymerization of NCA as reported previously (Tang et al., 2012). Briefly, before polymerization, L-aspartic acid-benzyl ester NCA was reacted with an excess of n-propylamine as the initiator. The product was precipitated in excess diethyl ether. The acquired white powder and L-phenylalanine NCA were dissolved in anhydrous DMF and stirred for 1 day at room temperature. The reaction mixture was then precipitated in diethyl ether and poly(L-aspartic acid-benzyl ester)–b-poly(L-phenylalanine) (PBAA–PPA) was obtained by filtration. To synthesize PAA–PPA, the PBAA–PPA was stirred in 1 mol/L NaOH aqueous solution and allowed to pass through a dialysis membrane. After 2 days of freeze-drying, PAA–PPA was collected (Structures of PAA–PPA, see Fig. 1).

2.5. Preparation of ATPR-M

A solution of ATPR (30 mg in 3 mL DMSO) was added dropwise to a stirred PAA–PPA solution (60 mg of PAA–PPA in 10 mL deionized water), then sonicated for 30 min (JY 92-IID ultrasonic processor, China) under cooling conditions. The mixed solution was subjected to dialysis against 2 L deionized water at 4 °C for 12 h. The micelle solution was then centrifuged at 3000 rpm for 10 min and passed through a 0.8 μm pore-sized microporous membrane and finally the filtrate was lyophilized. The final drug concentrations were adjusted to the required concentrations using normal saline before i.v. administration to mice and rats.

2.6. Pharmacokinetics of ATPR-M, ATPR CrmEL solution and ATPR solution in rats

Sixty SD rats (250 ± 20 g) were randomly assigned to ten groups for pharmacokinetic investigation. Each of the groups 1–8 received an intravenous (IV) injection of ATPR-M, and groups 9 and 10 received an IV injection of ATPR CrmEL solution (the solvent consisted of CrmEL and water with the volume ratio of 1:9) and ATPR solution (the solvent consisted of polyethylene glycol 400, propylene glycol, absolute ethyl alcohol and water with the volume ratio of 35:35:10:20), respectively. All the groups were injected in the femoral veins at an equivalent dose of 7 mg/kg (ATPR versus the body weight). Blood samples were collected from either the medial or lateral canthus of the rats at 0.083, 0.25, 0.5, 1, 2, 3, 4, 6, 8 and 10 h post-injection and centrifuged at 3000*g* for 10 min to obtain plasma which was stored at −20 °C. The ATPR concentrations were determined by HPLC. Pharmacokinetic parameters were obtained using the Kinetica 4.4 program.

2.7. Tissue distribution studies

Fifty-four male SD rats (250 ± 20 g) were used in the tissue distribution studies involving ATPR-M, ATPR solution and ATPR